Nature Conservation 24: 81–92 (2018)
doi: 10.3897/natureconservation.24.20650
http://natureconservation.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Comparative assessment of reproductive traits across different habitats in the endangered Webb's hyacinth (*Bellevalia webbiana* Parl.)

Giovanni Astuti[1], Gianni Bedini[1], Angelino Carta[1], Francesco Roma-Marzio[1], Alberto Trinco[1], Lorenzo Peruzzi[1]

1 *Department of Biology, Unit of Botany, University of Pisa, Via Derna, 1 56126, Pisa, Italy*

Corresponding author: *Giovanni Astuti* (vanni.astuti@gmail.com)

Academic editor: *I. Steffan-Dewenter* | Received 28 August 2017 | Accepted 22 December 2017 | Published 5 January 2018

http://zoobank.org/2C2262DF-DCBF-493A-AE68-A9C90107BBAE

Citation: Astuti G, Bedini G, Carta A, Roma-Marzio F, Trinco A, Peruzzi L (2018) Comparative assessment of reproductive traits across different habitats in the endangered Webb's hyacinth (*Bellevalia webbiana* Parl.). Nature Conservation 24: 81–92. https://doi.org/10.3897/natureconservation.24.20650

Abstract

To pursue a proper conservation of narrow endemic species, the knowledge of basic reproductive strategies is crucial to plan adequate conservation activities. One of the most evolutionarily relevant and threatened Italian endemic is the Webb's hyacinth (*Bellevalia webbiana* Parl.). As the reproductive behaviour of this species and its connection with human impact are currently unknown, the aim of this study was to characterise the reproductive traits of the Webb's hyacinth in contrasting habitats. All the 5 known richest populations across the species range were investigated. Their reproductive strategies were inferred by measuring inflorescence height, fruit set, seed set and *P/O* ratio. Reproductive features varied greatly amongst stands and also in relation to the degree of human disturbance. However, in all cases, seed sets showed low values. *P/O* values point towards full xenogamy and it is concluded that effective cross-pollination may be the main mode of sexual reproduction in *Bellevalia webbiana*. The reasons for the low reproductive performances may reside in pollen limitation, Allee effect and/or intrinsic reduced fertility of the species. Given this scenario, conservation efforts for Webb's hyacinth should focus on maintaining large and relatively dense populations, to guarantee some chance of *in situ* survival.

Keywords

conservation, Endangered, fruit set, IUCN, *P/O*, seed set

Copyright *Giovanni Astuti et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The Mediterranean basin is a well-known biodiversity hotspot (Myers et al. 2000) where, unfortunately, native plant diversity is highly threatened by environmental changes, notably human-induced changes in land use (Lee et al. 1995, McKinney 2002, Rossi et al. 2013). Within this context, Italy hosts about one third of the animal and half of the European plant taxa (Rossi et al. 2013). Amongst these taxa, the endemics are of particular importance (Siljak-Yakovlev and Peruzzi 2012), representing in Italy about 19% of the total vascular flora (Peruzzi et al. 2014). To pursue a proper conservation of these species, especially the narrow endemics, the knowledge of basic reproductive strategies is crucial in planning adequate conservation activities (Rossi et al. 2016). Indeed, as witnessed by IUCN categories (IUCN 2017), persistence of plant populations is intimately connected to generation time, so that life history studies usually feature an integrated approach uniting demography, reproductive biology and genetics in order to assess the persistence of plant populations in ecosystems (Ohara et al. 2006). Dealing with sexual species, reproductive success can tell a lot about plants' survival and responses to stress factors. However, in many narrow endemics, the paucity of available material (e.g. low number of individuals and flowers, inaccessible sites etc.) does not allow proper insight into reproductive performances, which also require extensive field works in order to take into account all the possible environmental and biological factors. Due to this impediment, the knowledge of the breeding system of threatened species is at least the first step for understanding to which threats they can most be subjected (e.g. habitat fragmentation, pollen limitation, Allee effect etc.).

Amongst the numerous Italian narrow endemic plants, arguably one of the most evolutionarily relevant and threatened, is the Webb's hyacinth (*Bellevalia webbiana* Parl., Asparagaceae, monocots; Chiarugi 1949, Borzatti von Loewenstern et al. 2013, Astuti et al. 2017). According to Gestri et al. (2010), the range of this bulbous perennial herb is restricted to an area of pre-Apennines (100–700 m a.s.l.) in Tuscany and Emilia-Romagna (Central Italy), with two disjunct population groups. Typical habitats for Webb's hyacinth are open fields and meadows, wood margins, olive groves and vineyards; during the last century, Webb's hyacinth disappeared from several historical localities due to human settlements (Gestri et al. 2010). For these reasons, this species is currently listed in The IUCN Red List of Threatened Species as Endangered (EN A2c) (Peruzzi and Carta 2011). Despite these contributions providing important information on the species' distribution, habitat and systematics, many ecological aspects, including the reproductive behaviour, are still unknown.

As a first attempt to fill this gap of knowledge, the aim of the present study is to characterise for the first time the reproductive traits of Webb's hyacinth. These traits were evaluated in contrasting habitats marked by different degrees of direct human impact and representative of the species' range. Particularly, the following questions were addressed: 1) which is the breeding system of the species? 2) Are reproductive performances different amongst populations?

Methods

Sampling sites

All the known richest populations of *Bellevalia webbiana* (five, each with $N > 50$ individuals) were included in the study (Table 1). These five populations also show different degrees and kinds of direct human impact: the habitat in Pratolino is an open olive grove near a much-frequented parking area, whose herbaceous-layer community is periodically cut. Uccellatoio represents a wood margin along a path, whose herbaceous layer is sporadically cut, but heavily dug by rooting of ungulates (especially wild boars). Tavarnuzze is an open herbaceous community surrounded by trees and shrubs and currently not managed by man. Faenza represents a wood margin along a path, within a private property (Apicoltura Lombardi), actively conserved by the owners. Finally, the population of Casola Valsenio occurs at the margins of a cultivated field. Despite the latter locality falling within the borders of the protected area "Parco Regionale della Vena del Gesso Romagnola", it certainly represents, together with Pratolino, one of the most humanly-impacted populations amongst those studied. The five study sites were visited three times during 2016: in February for a preliminary survey, in March for measuring and sampling inflorescences/flowers and in June for sampling fruits and seeds.

Plant traits

On 10–15 randomly selected individuals per population (those sampled in March differing from those sampled in June), data were collected on a number of reproductive traits. The following activities were carried out directly in the field in March 2016: *(a)* measurement of inflorescence height (cm) and *(b)* counting of flower number per inflorescence. Both these parameters are known to positively affect pollinator visits and pollen load (Pyke 1981, Andersson and Iwasa 1996, Donnelly et al. 1998), as well as herbivory impact (Sletvold and Grindeland 2008) and pre-dispersal seed predation

Table 1. Studied populations of *Bellevalia webbiana*.

Population	Municipality, Province, Region	Coordinates	Elevation (a.s.l.)
Pratolino	Vaglia, Florence, Tuscany	43.859745°N, 11.296976°E	464 m
Uccellatoio	Vaglia, Florence, Tuscany	43.859192°N, 11.293367°E	505 m
Tavarnuzze	Impruneta, Florence, Tuscany	43.720970°N, 11.226723°E	93 m
Faenza	Faenza, Ravenna, Emilia-Romagna	44.276015°N, 11.811606°E	147 m
Casola Valsenio	Casola Valsenio, Ravenna, Emilia-Romagna	44.242883°N, 11.671955°E	316 m

(Brody et al. 1997). In addition, two flowers showing not-yet dehiscing anthers were collected per raceme and their anthers were conserved under ethanol:glycerol 3:1 (v/v) solution in 1 ml Eppendorf vials. In June 2016, the fruit number per inflorescence *(c)* was counted in the field. Then, for each raceme, two fruits were randomly collected and conserved in separate small paper bags.

Later, in the laboratory, the two sampled flowers per individual were used to determine: *(d)* the ovule number (*O*) per ovary and *(e)* the estimated pollen-grain number per flower (*P*). The two sampled fruits per individual were used to count the seed number *(f)*. In order to estimate the pollen-grain number per flower, the protocol reported by Galloni et al. (2007) was followed, with slight modifications (Astuti et al. 2017): all the six anthers of each sampled flower, still under ethanol:glycerol solution in 1 ml Eppendorf vials, were sonicated for 1 min at 14 kHz by means of a Sonoplus Ultrasonic Homogeniser GM 2070. Just before the sonication, a few small grains of solid leucobasic fuchsin were added to the solution, in order to allow the staining of the pollen-grain walls, for easier counting. During the sonication, the vials were maintained in ice to avoid excessive warming and also retained there for 20–30 seconds after the sonication. Then, 1µl of homogenised solution was collected with a micropipette and placed on a microscope slide for pollen-grain counting. Each microscope slide was fully counted three times and, based on these three replicates, a mean total number of pollen-grains per sample was obtained. The estimation of the total pollen-grains number per flower was obtained by multiplying this number by 1000.

Starting from the above cited parameters, the fruit set [*(c)*/mean *(b)* for each population) and the seed set [*(f)*/*(d)*] were calculated for each individual. These two parameters are useful measures of reproductive performances, especially if related to pollination activity (Aguilar et al. 2006). In those cases where the fruit number of a certain individual was higher than the mean flower number for its population, the fruit set was adjusted to 1 by default. Finally, for each individual, the *P*/*O* ratio (Cruden 1977) was calculated. The variation of this ratio is correlated to the breeding strategy of a given angiosperm species: the lower the value, the more the plant is autogamous and vice-versa (Cruden 1977). In this case, this indirect method was adopted for assessing the breeding system due to relevant problems in flowers handling and manipulation (e.g. bagging experiments) in the field, due to their small size and unsuitable architecture.

Statistics

All the obtained data were analysed using PAST 3.14 software (Hammer et al. 2001, Hammer 2016). As all the considered variables were not normally distributed (after Shapiro-Wilk test) and lacked homogeneity of variance (after the Levene test), then the non-parametric Kruskal-Wallis test, complemented by Mann-Whitney pairwise comparison with Bonferroni correction, was used for comparing inflorescence height, number of flowers and number of fruits amongst populations. For the fruit set and seed set, χ^2 test was carried out. Only p values ≤ 0.01 have been considered significant.

Table 2. Reproductive parameters measured in the five studied populations of *Bellevalia webbiana*. The measurements are reported as mean value ± standard deviation.

	Pratolino	Uccellatoio	Tavarnuzze	Faenza	Casola
Inflorescence height (cm)	17.24 ± 4.11	24.85 ± 8.02	29.65 ± 5.05	51.84 ± 21.73	27.96 ± 7.79
Number of flowers	44.13 ± 10.06	32.57 ± 9.09	40.60 ± 12.47	62.15 ± 17.77	33.60 ± 6.58
Number of fruits	0	16.00 ± 9.79	13.93 ± 7.59	49 ± 19.27	16.53 ±11.07
Number of ovules	6	6	6	5.93 ± 0.26	6
Number of estimated pollen grains	28,261.90 ± 12,720.79	33,928.57 ± 11,090.57	21,130.95 ± 7,445.44	28,965.52 ± 7,533.87	23,000.71 ± 11,279.55
Number of seeds	0	2.17 ± 1.32	2.20 ± 1.45	2.57 ± 1.41	2.33 ± 1.12
Fruit Set	0	0.50 ± 0.27	0.34 ± 0.19	0.74 ± 0.25	0.47 ± 0.26
Seed Set	0	0.36 ± 0.16	0.37 ± 0.17	0.43 ± 0.19	0.39 ± 0.13
P/O	4,710.42 ± 2,120.05	5,654.76 ± 1,848.35	3,521.82 ± 1,240.96	4,827.58 ± 1,255.59	3,833.32 ± 1,880

Results

All the measured values are summarised in Table 2. Generally, the Faenza population shows values of inflorescence height (Figure 1), flower number and fruit number (Figure 2) significantly higher (Mann-Whitney test with Bonferroni correction).

Some more complex relationships concerning statistical differences amongst populations were found for the estimated number of pollen-grains per flower (see also Figure 3) and, given that the ovule number remained almost constant, consequently also for *P/O* values (Table 2).

Due to heavy cutting of all the individuals of the Pratolino population before fructification time, the number of fruits and seeds produced there dropped to zero (Figures 3, 4). χ^2 revealed significant differences amongst the remaining four populations concerning the fruit set, whereas no significant differences concerning seed number and seed set were found (Figure 4). χ^2 test revealed significant differences in the fruit set for all the pairwise comparisons, except between Uccellatoio and Casola. The Faenza population showed the highest fruit set, whereas Tavarnuzze the lowest. In all the populations, the mean seed set value was below 0.5 (Table 2, Figure 4).

Discussion

It was possible to highlight that, in *B. webbiana,* the ovule number is almost constant, with 2 ovules in a row for each of the three ovary locules. This confirms previous reports for the genus (Speta 1998). The *P/O* values of the studied populations, despite some differences (Table 2), all fall in a range of values reported by Cruden (1977) as typical of fully xenogamous species. In *Bellevalia,* the vegetative propagation was never reported (Feinbrun 1940, Speta 1998). However, in a plant coming from Casola Valsenio and cultivated in laboratory, the production of a small bulbil detaching from the main bulb was observed. Accordingly, *B. webbiana* can occasionally reproduce by means of vegetative propagation.

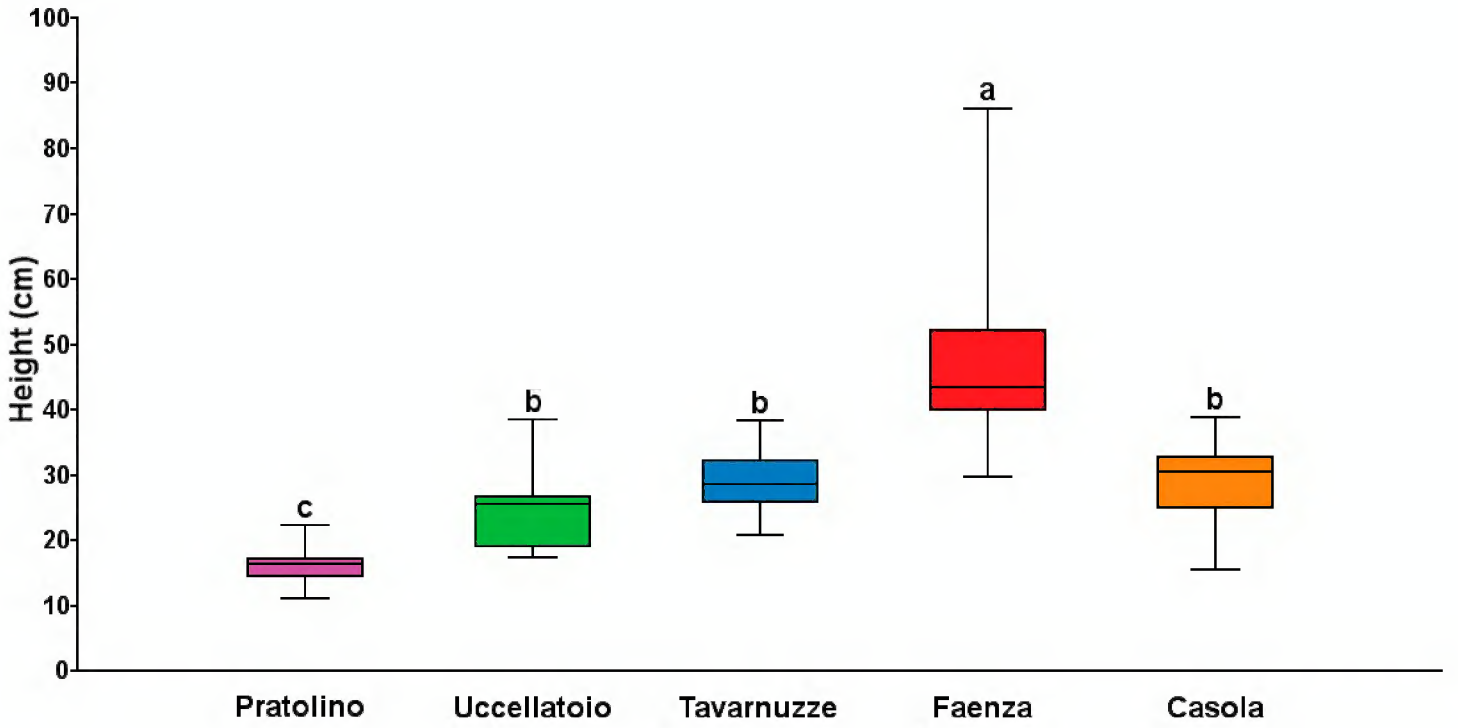

Figure 1. Boxplot of the inflorescence height (cm) in the studied *Bellevalia webbiana* populations. Different letters indicate statistically significant differences at 0.01 level.

The Faenza population, the only one actively conserved, shows several significant differences from other populations, concerning a higher reproductive effort (longer inflorescences with more flowers, more fruits and a higher fruit set; Figure 1, Table 2). This population is in close proximity to beehives and bees have been observed by the authors on Webb hyacinth's flowers during this research. However, despite the higher number of fruits produced and fruit set (Table 2, Figure 2), the seed set showed no significant differences compared to other populations (Tavarnuzze, Uccellatoio, Casola Valsenio; Figure 4). Although the effective contribution of bees in alleviating the possible pollen limitation was not quantified, the not-significantly higher reproductive success in this population may point towards other general problems, such as inbreeding depression or other intrinsic biological problems of the species.

On the other hand, there are the critical situations of Pratolino and Casola Valsenio. The reiterated periodical cutting in Pratolino population may easily explain the reduced size of inflorescences (Figure 1), due to lower nutrients stored in the bulb for the following year (Muller 1976, Werger and Huber 2006). Moreover, the cutting is, of course, the cause of the null reproductive outcome for this population. *Bellevalia webbiana* is perennial and long-living (a generation is estimated to be around 40 years by Gestri et al. 2010) and it is well known in literature that, in long-lived perennials, annual variation of reproductive success might be of minor importance (see, for instance, the study by Hoernemann et al. 2012 on *Muscari tenuiflorum* Tausch, a species from a genus phylogenetically close to *Bellevalia*). However, despite this, the reiterated reduction or absence of sexual reproduction might represent a relevant problem for the long-term survival of a species (Rathcke and Jules 1993). A general weakening of the individuals in the Casola Valsenio population may explain the slightly lower number of pollen-grains produced per flower (Figure 3). Indeed, still in 2010, this population was

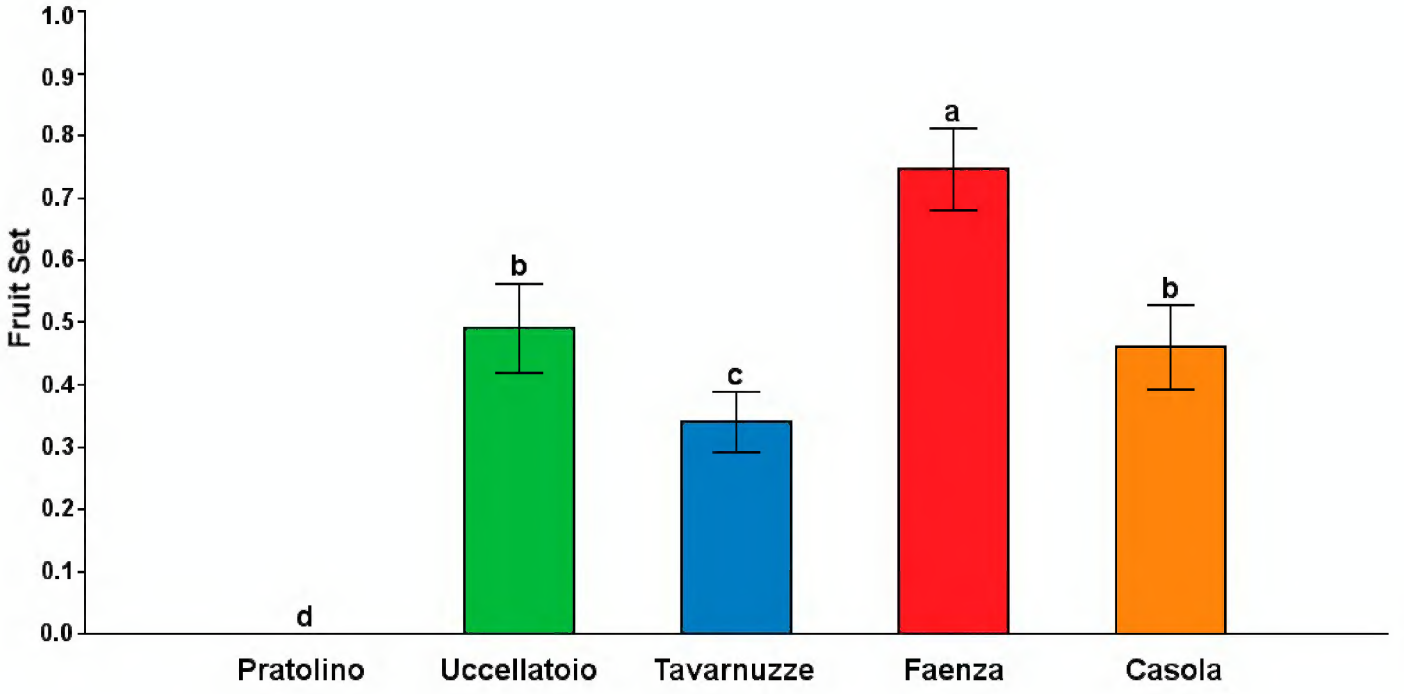

Figure 2. Box-chart of fruit set amongst the studied *Bellevalia webbiana* populations (confidence interval, 95%). Different letters indicate statistically significant differences at 0.01 level.

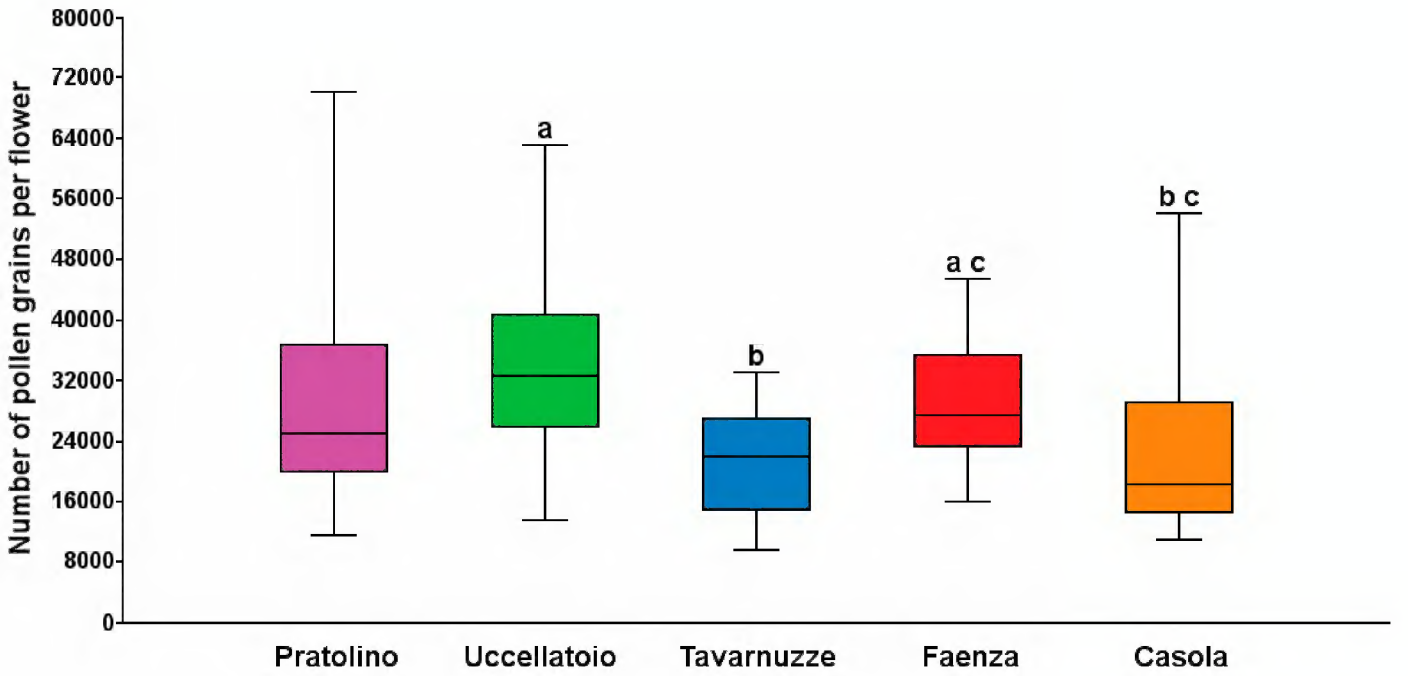

Figure 3. Boxplot of the estimated pollen grain number per flower in the studied *Bellevalia webbiana* populations. Different letters indicate statistically significant differences at 0.01 level.

made up of around 300 individuals (Gestri et al. 2010), but a very recent, improper, change in soil use decimated the population to a few tens of individuals at the margins and in-between the clumps of what is currently a ploughed field.

On the other hand, all the studied populations showed low seed set values in the 2016 flowering season (Figure 4). This result may point towards pollen limitation phenomena (Burd 1994). This appears likely, also considering the relatively early flowering period for this species, which often causes an unpredictable pollinator service

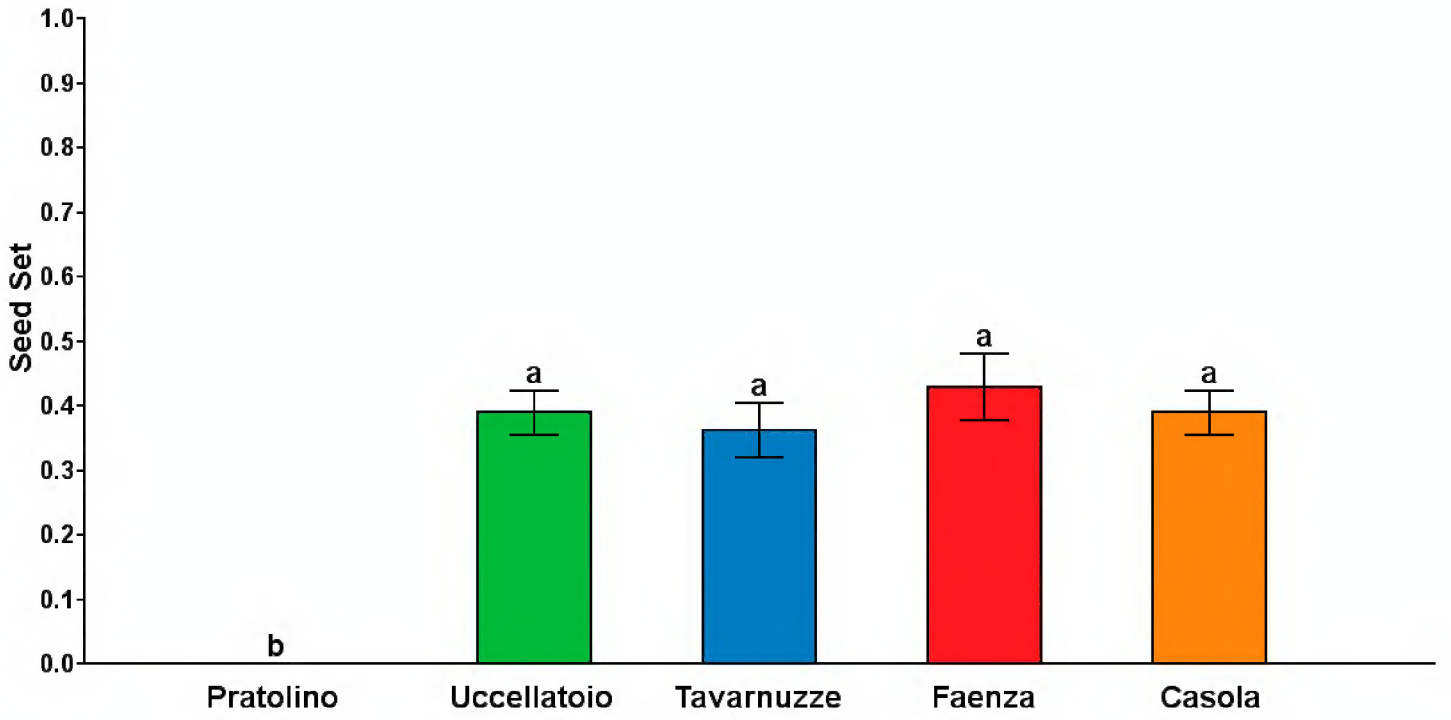

Figure 4. Box-chart of seed set in the studied *Bellevalia webbiana* populations (confidence interval, 95%). Different letters indicate statistically significant differences at 0.01 level.

(McCall and Primack 1992, Baker et al. 2000). Given that Webb's hyacinth seems xenogamous, it may also suffer some density-dependent fitness reduction, i.e. Allee effect (Courchamp 1999, Ashman et al. 2004), as observed in many other herbaceous entomophilous species (e.g. Kunin 1997, Schleuning et al. 2009, Hornemann et al. 2012). Another possible explanation for the low general reproductive performance is the occurrence of inbreeding depression due to isolation and fragmentation of the populations. Deleterious alleles, fixed by inbreeding depression, may easily cause abortion of fruits and/or seeds, especially during the earlier developmental stages (Wiens 1984, Rathcke and Jules 1993, Baker et al. 2000, Schleuning et al. 2007), or cause the production of low-quality pollen (Ashman et al. 2004). These phenomena could even more markedly affect the reproductive performance of smaller populations other than the five studied here, which are the richest currently known in terms of number of individuals. It is also noteworthy to state that the polyploid origin of this species is well documented (Chiarugi 1949, Borzatti von Loewenstern et al. 2013 and literature cited therein), so that intrinsic fertility problems for the Webb's hyacinth, due to its peculiar genomic constitution cannot be excluded. It is indeed well known that polyploidy can significantly reduce fertility in sexual reproduction (Levin 2002). Despite this, Capineri et al. (1979) documented for this species a regular meiosis with bivalents formation and this may lend support to different explanations for the low seed set (e.g. pollen limitation and/or Allee effect).

This study was conducted within a single year, providing thus a partial view of the reproductive behaviour of the species and its connection with human impact. However, these preliminary results already pointed towards urgent conservation issues, as habitat deterioration in several populations is progressing very rapidly. Given this scenario, conservation efforts for Webb's hyacinth should be devoted to

maintain large and relatively dense populations, in order to guarantee some chance of *in situ* survival. The direct human impact on the sites (e.g. cutting etc.) should be allowed only after the seed dispersal, as this seems the prevalent reproductive method available to this species.

Further studies are necessary in order to check the reproductive performances in the medium-long period and to experimentally verify the hypotheses of possible (co-) occurrence of pollen limitation, Allee effect and/or intrinsic fertility problems of the species. In addition, it will also be useful to investigate the vegetative traits of Webb's hyacinth in relation to (especially human-induced) environmental changes. In the meantime, active *ex situ* conservation protocols have been established, by means of seeds stored in the Pisa Germplasm Bank (Italy) and in the Millennium Seed Bank, Kew Gardens (London, UK), complemented by propagation and cultivation in the Botanic Garden of the University of Pisa.

Acknowledgements

Mauro Lombardi from "Apicoltura Lombardi" is gratefully acknowledged, for logistic help and permission to work within his properties. We also thank the Director of the "Parco Regionale della Vena del Gesso Romagnola", for his support and permission to carry out this study. This work was funded by the "Progetto di Ricerca di Ateneo" (PRA) of the University of Pisa, under grant number PRA_2016_1.

References

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9: 968–980. https://doi.org/10.1111/j.1461-0248.2006.00927.x

Andersson M, Iwasa Y (1996) Sexual selection. Trends in Ecology and Evolution 11: 53–58. https://doi.org/10.1016/0169-5347(96)81042-1

Ashman T, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85(9): 2408–2421. https://doi.org/10.1890/03-8024

Astuti G, Brullo S, Domina G, El Mokni R, Giordani T, Peruzzi L (2017) Phylogenetic relationships among tetraploid species of *Bellevalia* (Asparagaceae) endemic to south central Mediterranean. Plant Biosystems 151(6): 1120–1128. https://doi.org/10.1080/11263504.2017.1320308

Astuti G, Roma-Marzio F, D'Antraccoli M, Bedini G, Carta A, Sebastiani F, Bruschi P, Peruzzi L (2017) Conservation biology of the last Italian population of *Cistus laurifolius* (Cistaceae): demographic structure, genetics and reproductive success. Nature Conservation 22: 169–190. https://doi.org/10.3897/natureconservation.22.19809

Baker AM, Barrett SCH, Thompson JD (2000) Variation of pollen limitation in the early flowering Mediterranean geophytes *Narcissus assoanus* (Amaryllidaceae). Oecologia 124: 529–535. https://doi.org/10.1007/s004420000417

Barrios-García MN, Ballari SA (2012) Impact of wild boar (*Sus scrofa*) in its introduced and native range: a review. Biological Invasions 14: 2283–2300. https://doi.org/10.1007/s10530-012-0229-6

Borzatti von Loewenstern A, Giordani T, Astuti G, Andreucci A, Peruzzi L (2013) Phylogenetic relationship of Italian *Bellevalia* species (Asparagaceae), inferred from morphology, karyology and molecular systematics. Plant Biosystems 147: 776–787. https://doi.org/10.1080/11263504.2013.829884

Brody AK, Mitchell RJ (1997) Effects of experimental manipulation of inflorescence size on pollination and predispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. Oecologia 110: 86–94. https://doi.org/10.1007/s004420050136

Burd M (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Botanical Review 60(1): 83–139. https://doi.org/10.1007/BF02856594

Capineri R, D'Amato G, Marchi P (1979) Numeri Cromosomici per la Flora Italiana: 534–583. Informatore Botanico Italiano 10(3)(1978): 421–465.

Chiarugi A (1949) Saggio di una revisione cito-sistematica della flora italiana I Il tetraploidismo della *Bellevalia webbiana* Parl e il suo diritto di cittadinanza nella flora italiana. Caryologia 1(3): 362–376. https://doi.org/10.1080/00087114.1949.10797519

Courchamp F, Clutton-Brock T, Grenfell B (1999) Inverse density dependence and the Allee effect. Trends in Ecology and Evolution 10: 405–410. https://doi.org/10.1016/S0169-5347(99)01683-3

Cruden RW (1977) Pollen-ovule ratio: a conservative indicator of breeding systems in flowering plants. Evolution 31(1): 32–46. https://doi.org/10.1111/j.1558-5646.1977.tb00979.x

Donnelly SE, Lortie CJ, Aarsen LW (1998) Pollination in *Verbascum thapsus* (Scrophulariaceae): the advantage of being tall. American Journal of Botany 85: 1618–1625. https://doi.org/10.2307/2446490

Feinbrun N (1940) A monographic study on the genus *Bellevalia* Lapeyr (karyology, taxonomy, geography) (continued). Palestine Journal of Botany 1(4): 336–409.

Galloni M, Podda L, Vivarelli D, Cristofolini G (2007) Pollen presentation, pollen-ovule ratios, and other reproductive traits in Mediterranean Legumes (Fam Fabaceae - Subfam Faboideae). Plant Systematics and Evolution 266: 147–164. https://doi.org/10.1007/s00606-007-0526-1

Gestri G, Alessandrini A, Sirotti N, Carta A, Peruzzi L (2010) Contributo alla conoscenza della flora vascolare endemica di Toscana ed aree contermini 2 *Bellevalia webbiana* Parl (Asparagaceae). Informatore Botanico Italiano 42: 449–455.

Hammer Ø (2016) PAST version 3.14. http://folkuiono/ohammer/past/

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9.

Hornemann G, Weiss G, Durka W (2012) Reproductive fitness, population size and genetic variation in *Muscari tenuiflorum* (Hyacinthaceae): the role of temporal variation. Flora 207: 736–743. https://doi.org/10.1016/j.flora.2012.07.005

IUCN (2017) Guidelines for using the IUCN red list categories and criteria. Version 13. Prepared by the Standards and petitions Subcommittee. http://cmsdocs.s3.amazonaws.com/RedListGuidelines.pdf

Levin DA (2002) The role of chromosomal change in plant evolution. University Press, Oxford, 240 pp.

Lee H, Carr JL, Lankerani A (1995) Human disturbance and natural habitat: a biome level analysis of a global data set. Biodiversity and Conservation 4: 128–155. https://doi.org/10.1007/BF00137781

Kunin WE (1997) Population size and density effects in pollination: pollinators foraging and plant reproductive success in experimental arrays of *Brassica kaber*. Journal of Ecology 85: 225–234. https://doi.org/10.2307/2960653

McCall C, Primack RB (1992) Influence of flower characteristics, weather, time of day and season on insect visitation rates in three plant communities. American Journal of Botany 79: 434–442. https://doi.org/10.2307/2445156

McKinney ML (2002) Urbanization, Biodiversity, and Conservation. BioScience 52(10): 883–890. https://doi.org/10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2

Muller RN (1978) The phenology, growth and ecosystem dynamics of *Erythronium americanum* in the northern hardwood forest. Ecological Monograph 48: 1–20. https://doi.org/10.2307/2937357

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501

Ohara M, Tomimatsu H, Takeda T, Kawano S (2006) Importance of life history studies for conservation of fragmented populations: a case study of the understory herb, *Trillium camschatcense*. Plant Species Biology 21: 1–12. https://doi.org/10.1111/j.1442-1984.2006.00145.x

Peruzzi L, Carta A (2011) *Bellevalia webbiana*. The IUCN Red List of Threatened Species 2011: eT195349A8957996. http://dxdoiorg/102305/IUCNUK2011-2RLTST195349A8957996en

Peruzzi L, Conti F, Bartolucci F (2014) An inventory of vascular plants endemic to Italy. Phytotaxa 168(1): 1–75. https://doi.org/10.11646/phytotaxa.168.1.1

Pyke GH (1981) Effects of inflorescence height and number of flowers per inflorescence on fruit-set in waratahs (*Telopea speciosissima*). Australian Journal of Botany 29: 419–424. https://doi.org/10.1071/BT9810419

Rathcke BJ, Jules ES (1993) Habitat fragmentation and plant-pollinator interaction. Current Science 65(3): 273–277.

Rossi G, Montagnani C, Gargano D, Peruzzi L, Abeli T, Ravera S, Cogoni A, Fenu G, Magrini S, Gennai M, Foggi B, Wagensommer RP, Venturella G, Blasi C, Raimondo FM, Orsenigo S (2013) Lista Rossa della Flora Italiana 1 Policy Species e altre specie minacciate. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma, 1–58.

Rossi G, Orsenigo S, Montagnani C, Fenu G, Gargano D, Peruzzi L, Wagensommer RP, Foggi B, Bacchetta G, Domina G, Conti F, Bartolucci F, Gennai M, Ravera S, Cogoni A, Magrini S, Gentili R, Castello M, Blasi C, Abeli T (2016) Is legal protection enough to ensure plant conservation? Italian red lists as a case study. Oryx 50(3): 431–436. https://doi.org/10.1017/S003060531500006X

Schleuning M, Niggemann M, Becker U, Matthies D (2007) Negative effect of degradation and fragmentation on the declining grassland plant *Trifolium montanum*. Basic Applied Ecology 10: 61–69. https://doi.org/10.1016/j.baae.2007.12.002

Siljak-Yakovlev S, Peruzzi L (2012) Cytogenetic characterization of the endemics: past and future. Plant Biosystems 146: 694–702.

Sletvold N, Grindeland JM (2008) Floral herbivory increases with inflorescence size and local plant density in *Digitalis purpurea*. Oecologia 34: 21–25. https://doi.org/10.1016/j.actao.2008.03.002

Speta F (1998) Hyacinthaceae. In: Kubitzki K (Ed.) The families and genera of vascular plants: flowering plants, Monocotyledons Lilianae (except Orchidaceae) 3. Springer-Verlag, Berlin, 261–285. https://doi.org/10.1007/978-3-662-03533-7_35

Werger MJA, Huber H (2006) Tuber size variation and organ preformation constrain growth responses of a spring geophyte. Oecologia 147: 396–405. https://doi.org/10.1007/s00442-005-0280-4

Wiens D (1984) Ovules survivorship, brood size, life history, breeding system, and reproductive success in plants. Oecologia 64: 47–53. https://doi.org/10.1007/BF00377542